

Fig. 1

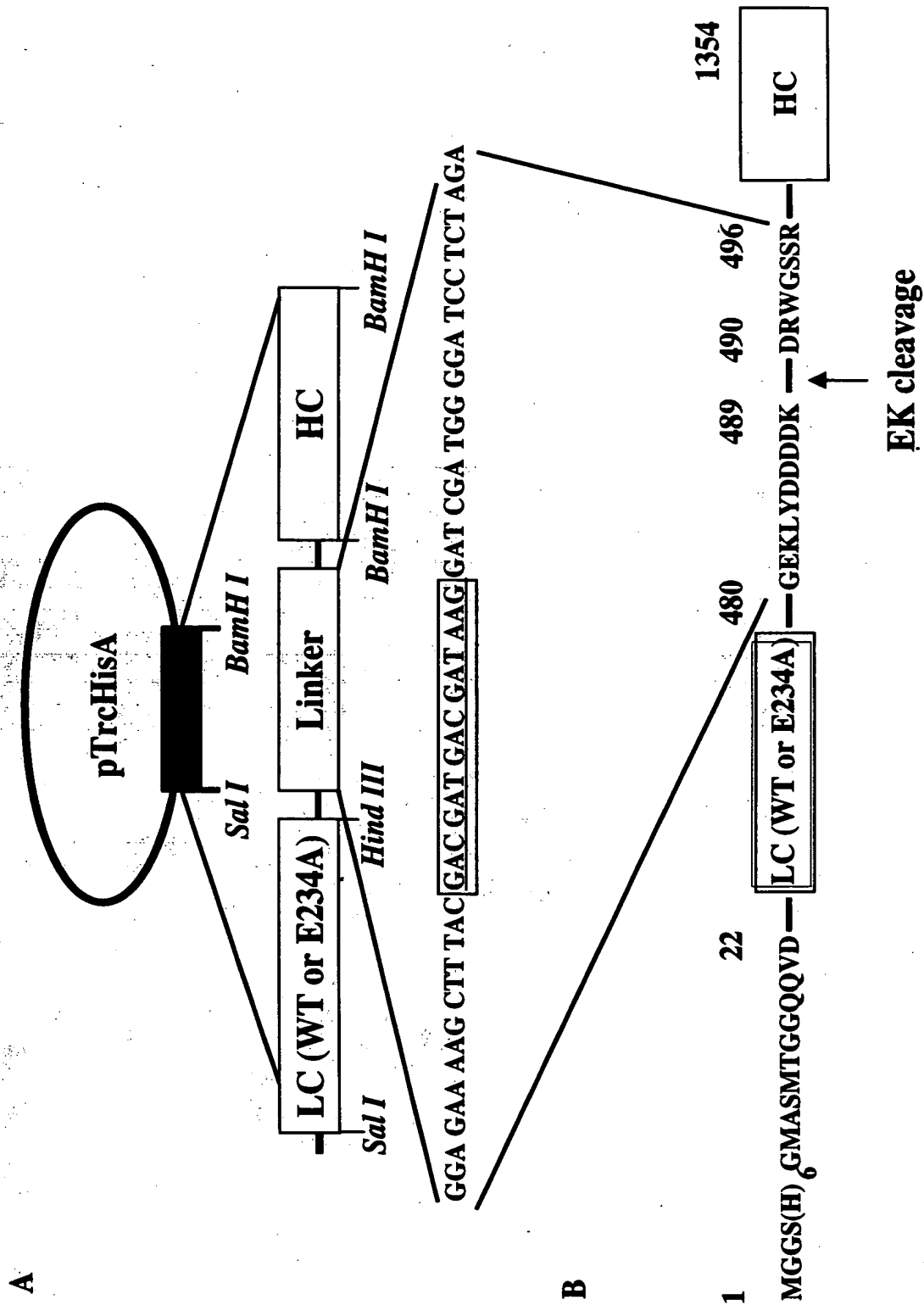


Fig. 2

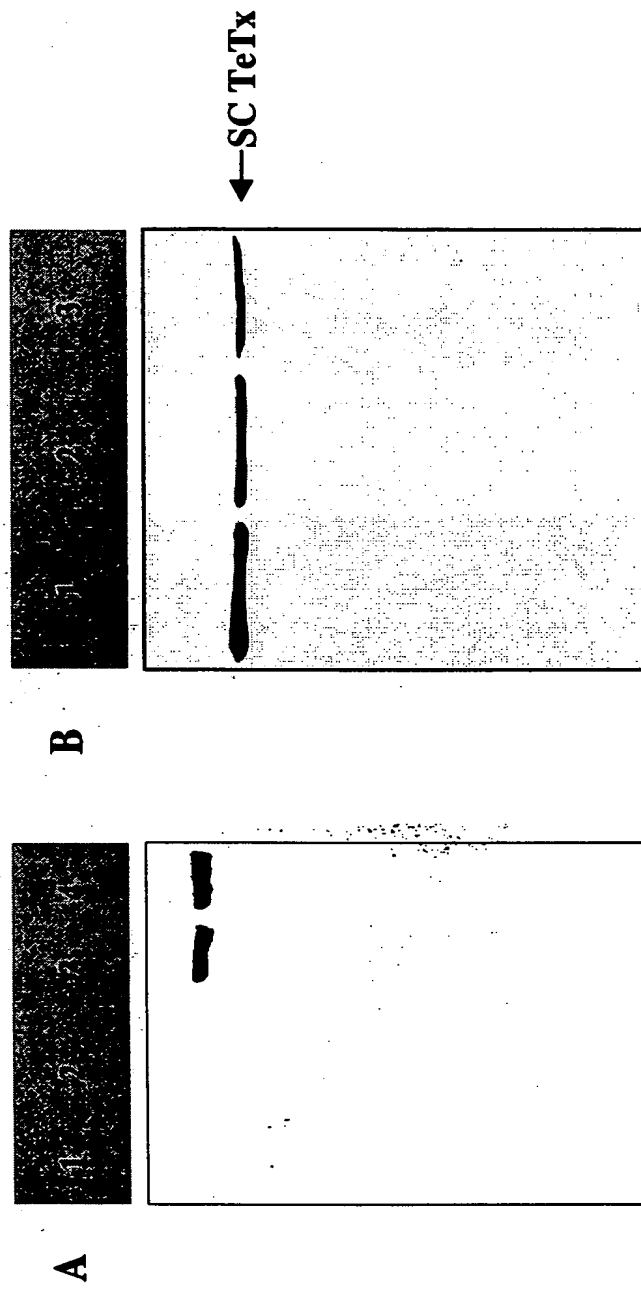


Fig. 3

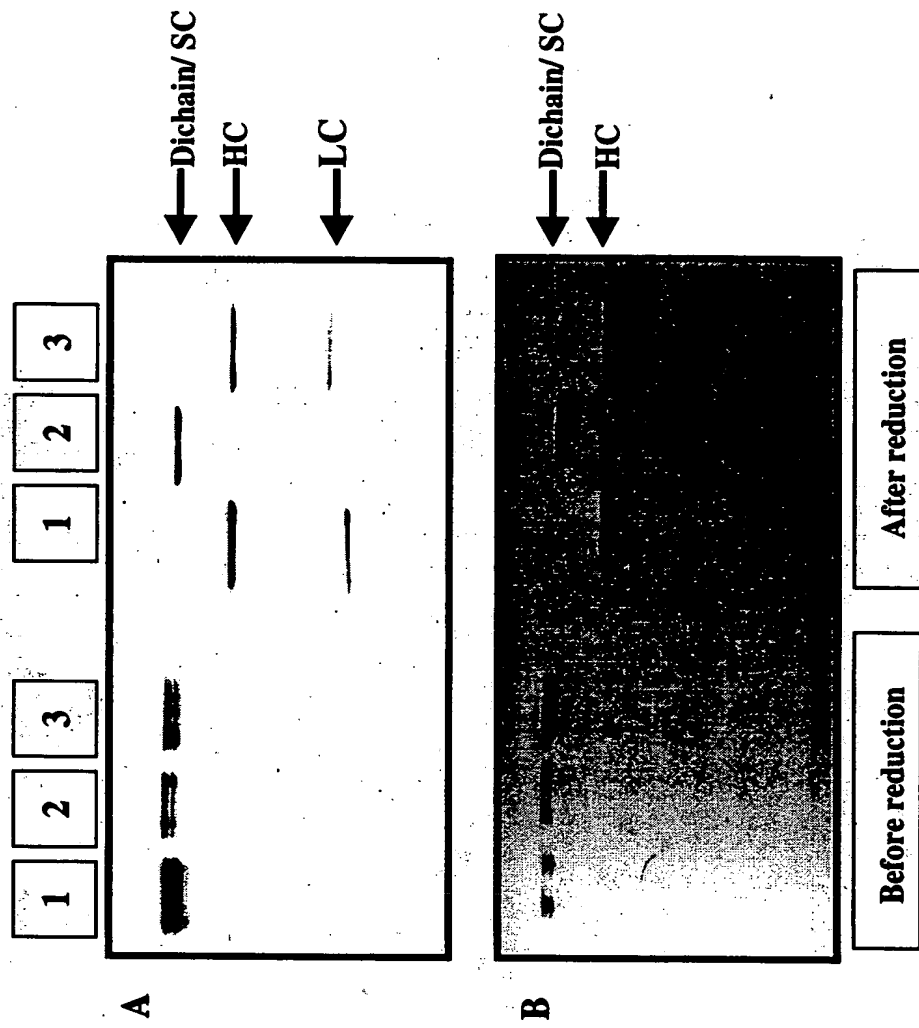
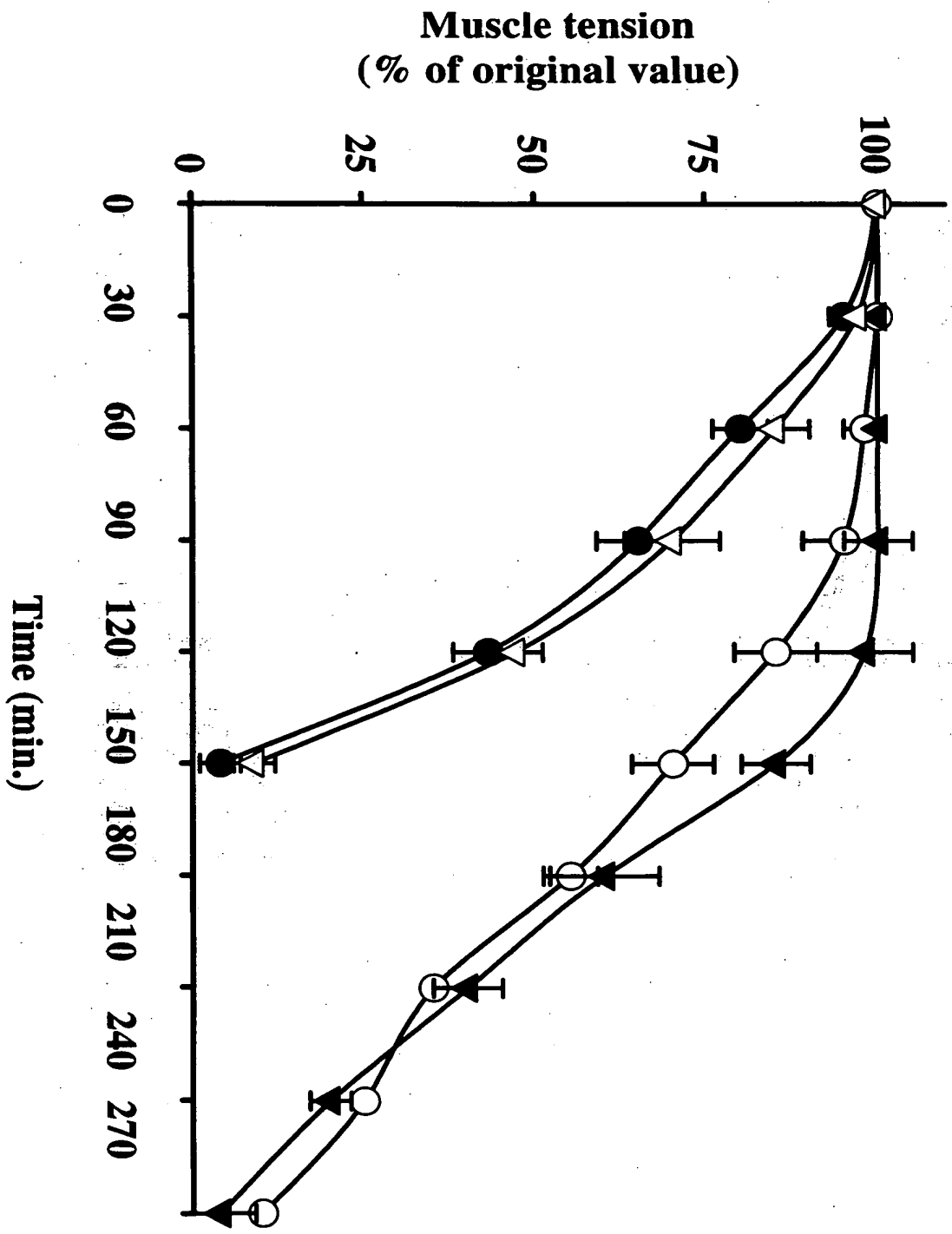


Fig. 4



00644692-082500

Fig. 5

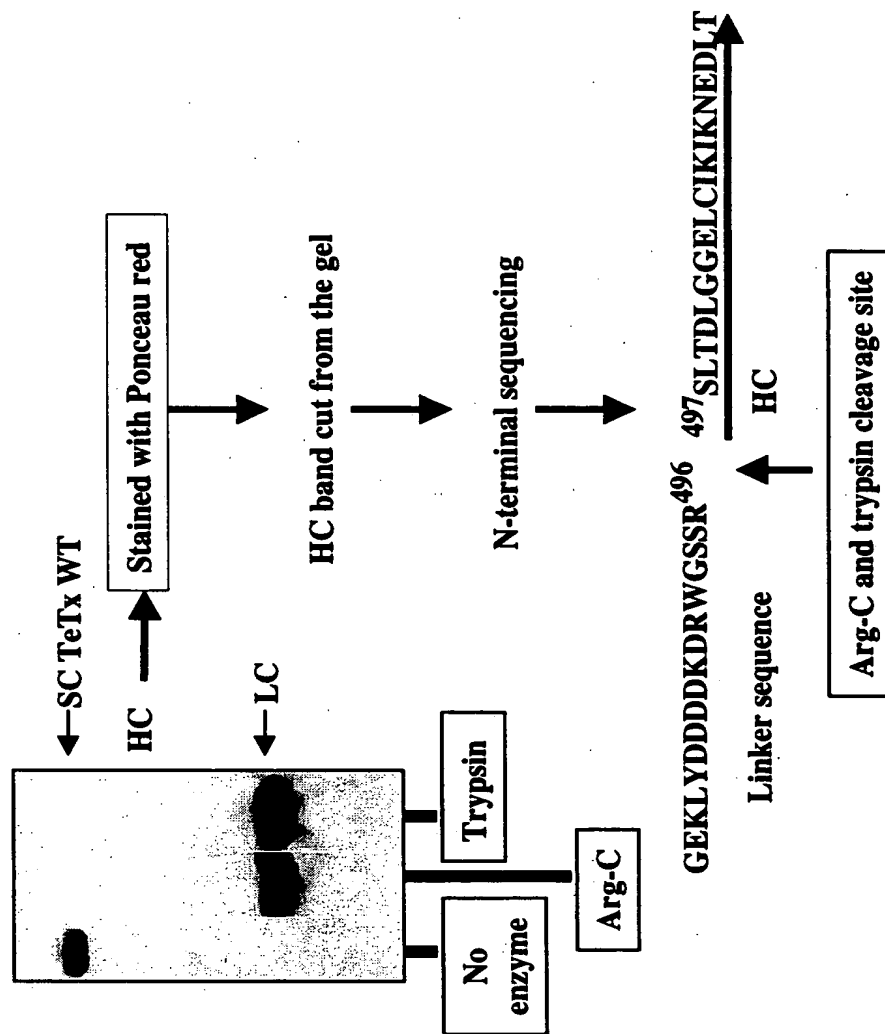


Fig. 6

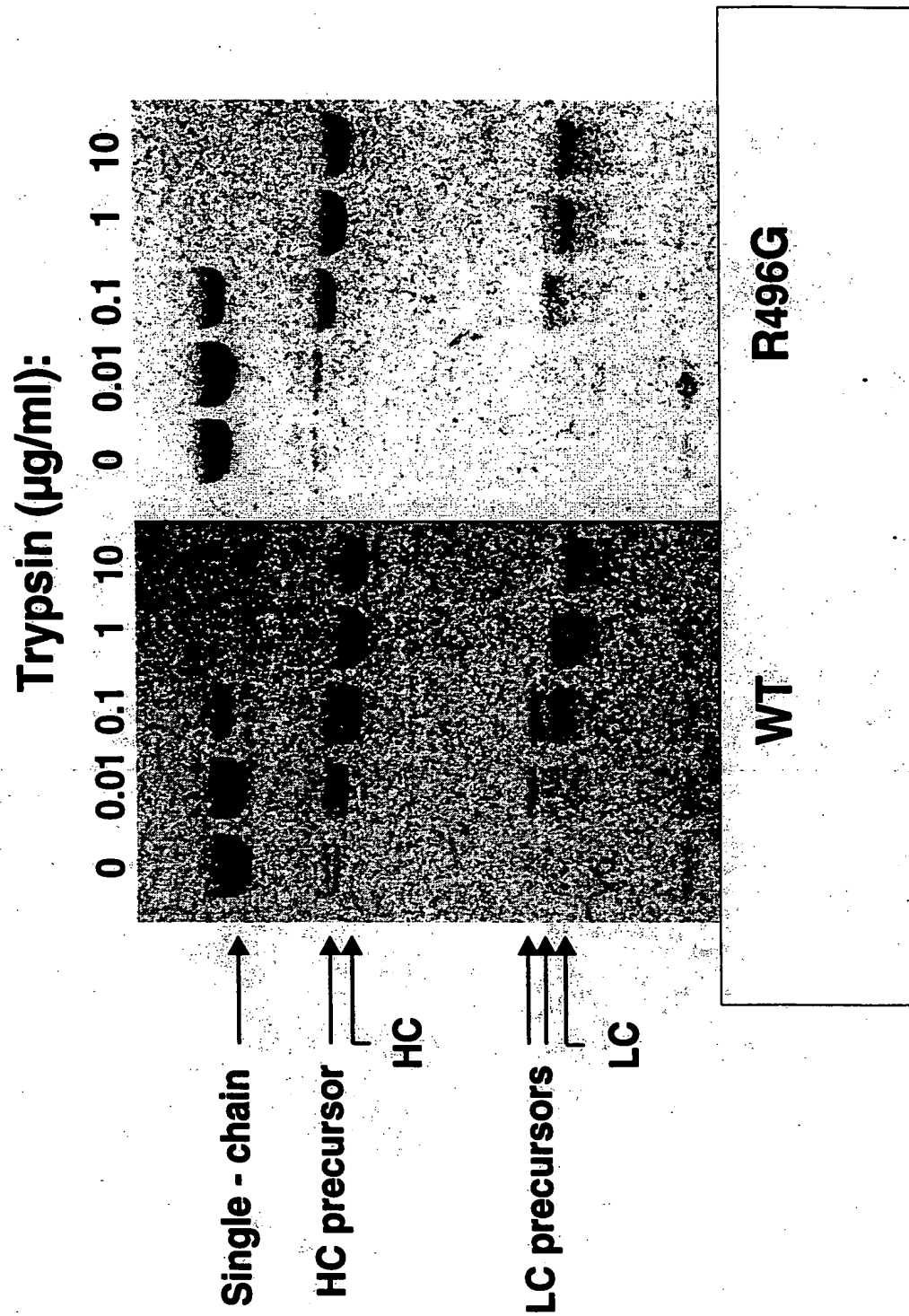


Fig. 7

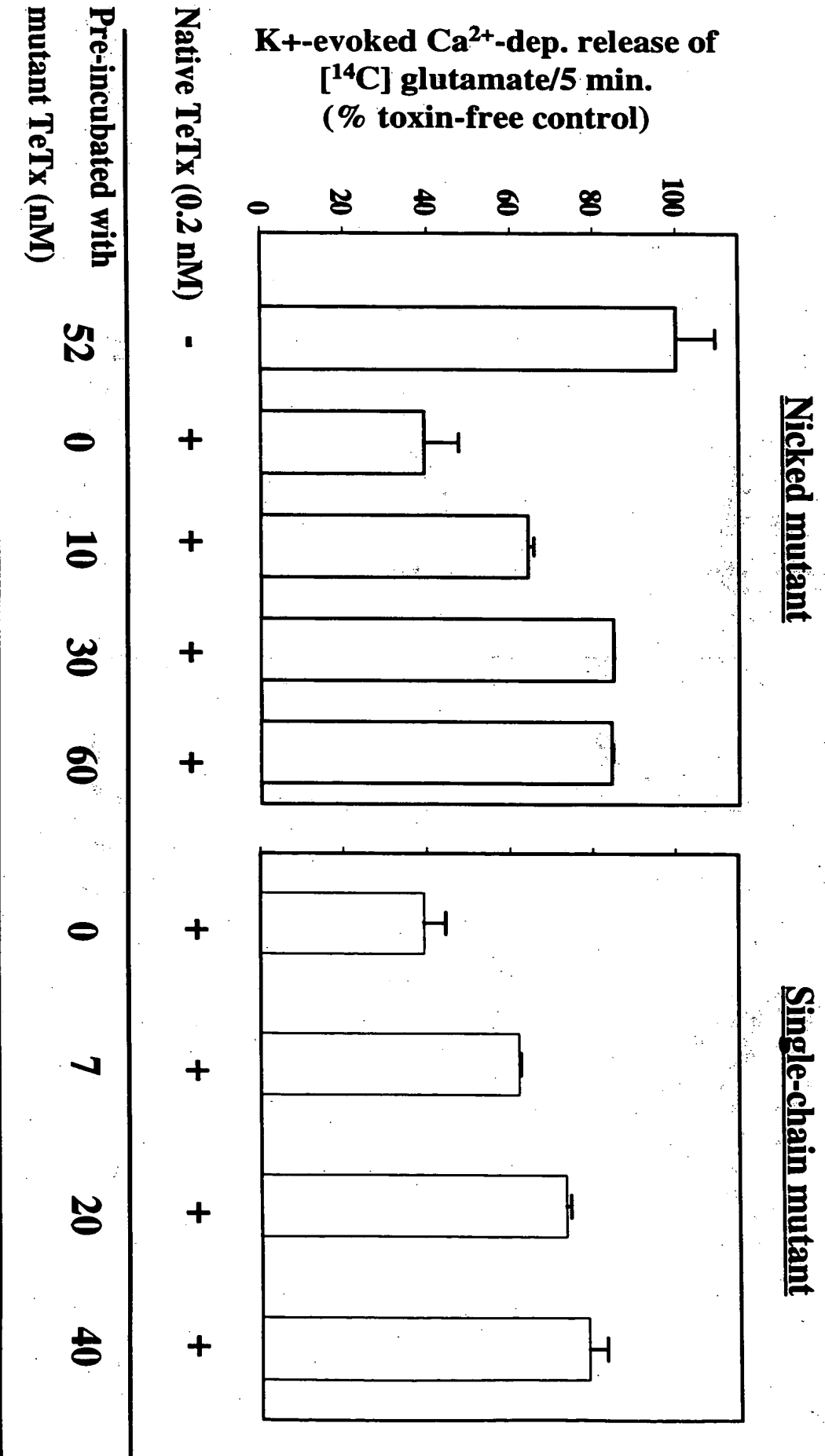
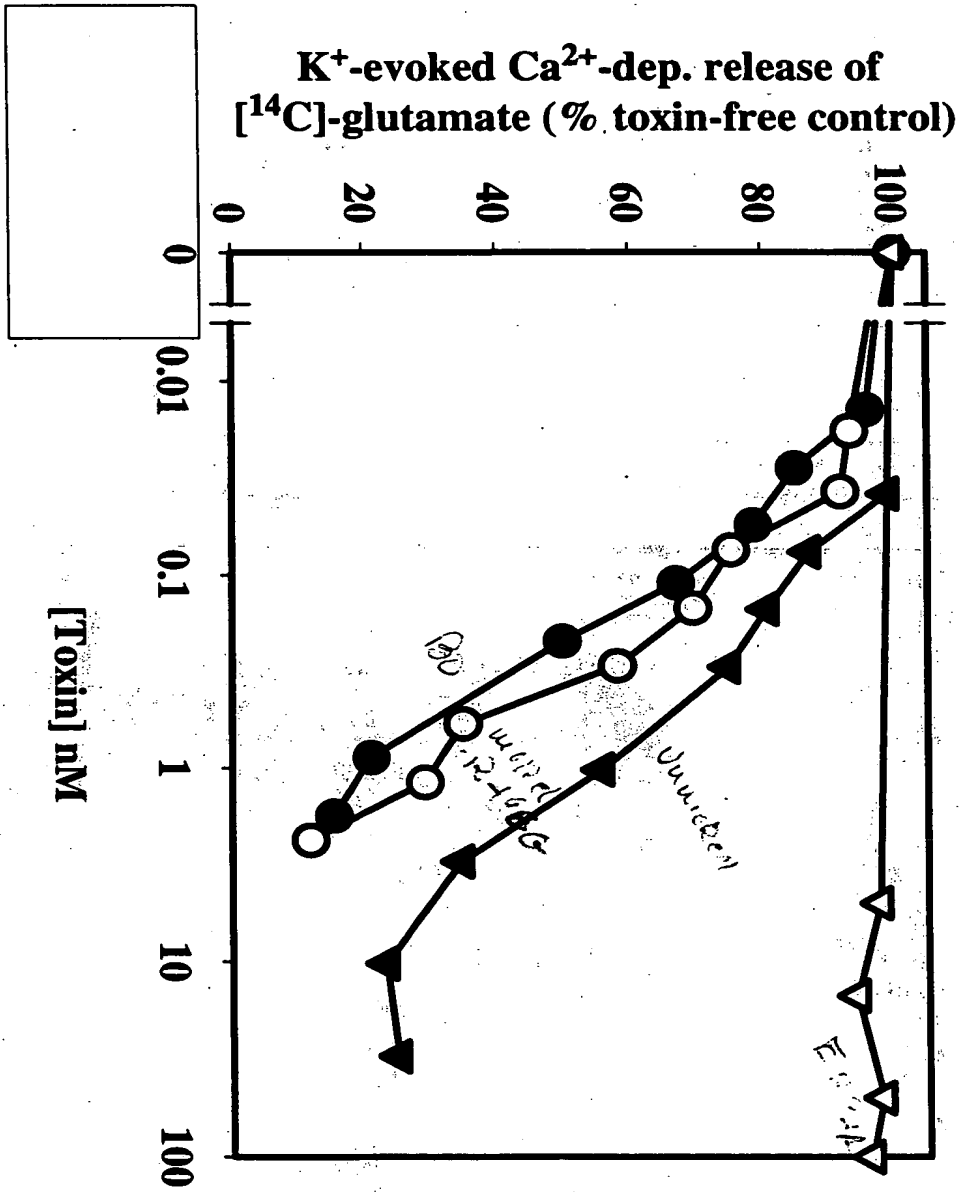
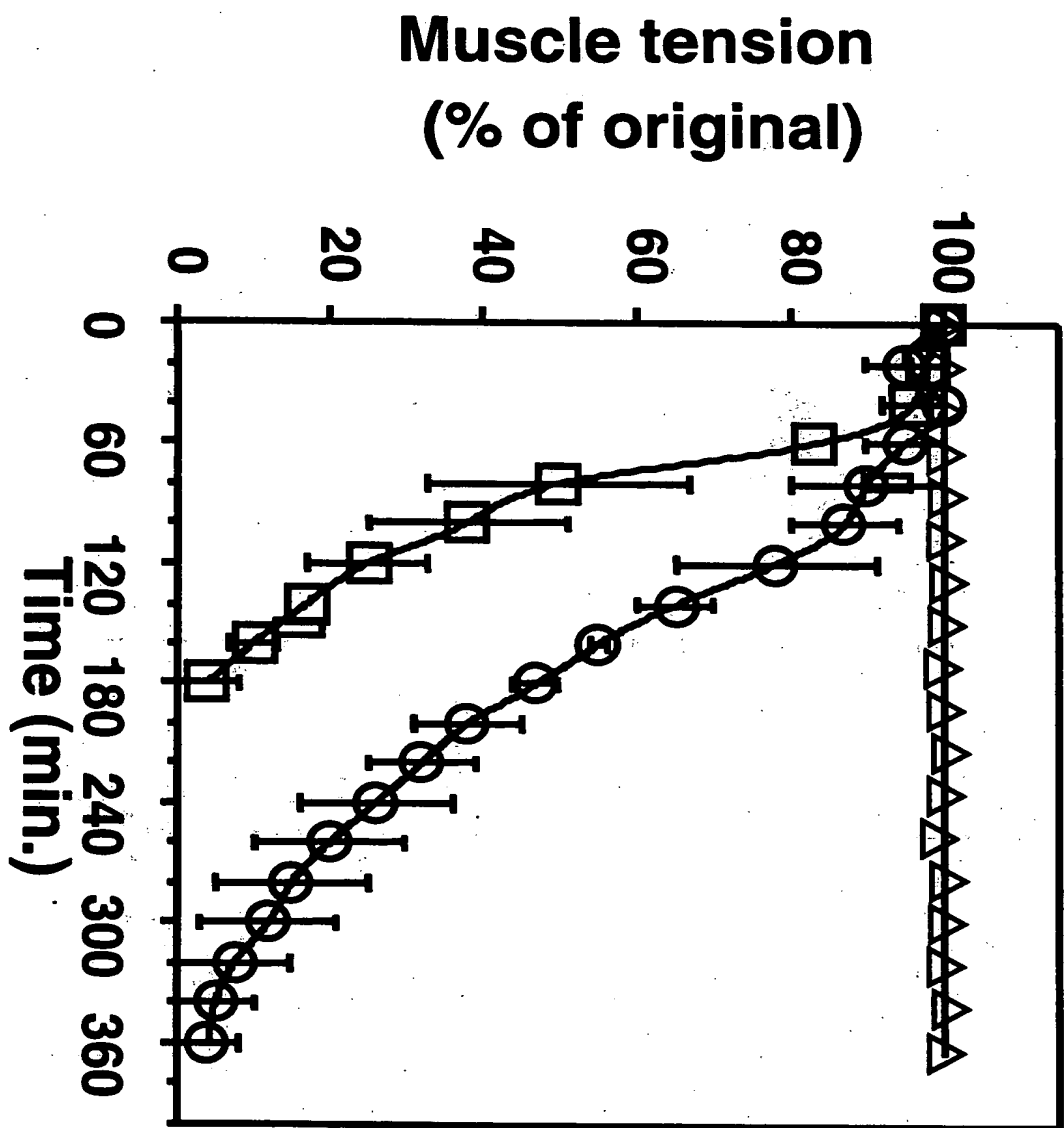


Fig. 8



00548592-082500

Fig. 9

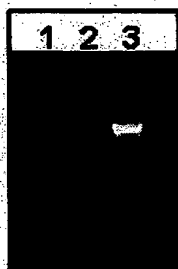


09648592-082500

Fig. 10

Clostridium botulinum type E
(strain beluga) chromosomal DNA

PCR with a proof reading
thermopolymerase



1: Markers
2: Negative control
3: Sample

3.5-kB DNA fragment purified
and cloned into BamHI and PstI
sites of pQE30 expression vector



ATG	AGA	GGA	TCG	CAT	CAC	CAT	CAC	CAT	CAC	GGA	TCC	CCA	AAA	ATT	AAT	AGT	TTT
M	R	G	S	H	H	H	H	H	H	G	S	P	K	I	N	S	T
Start										Bam HI							

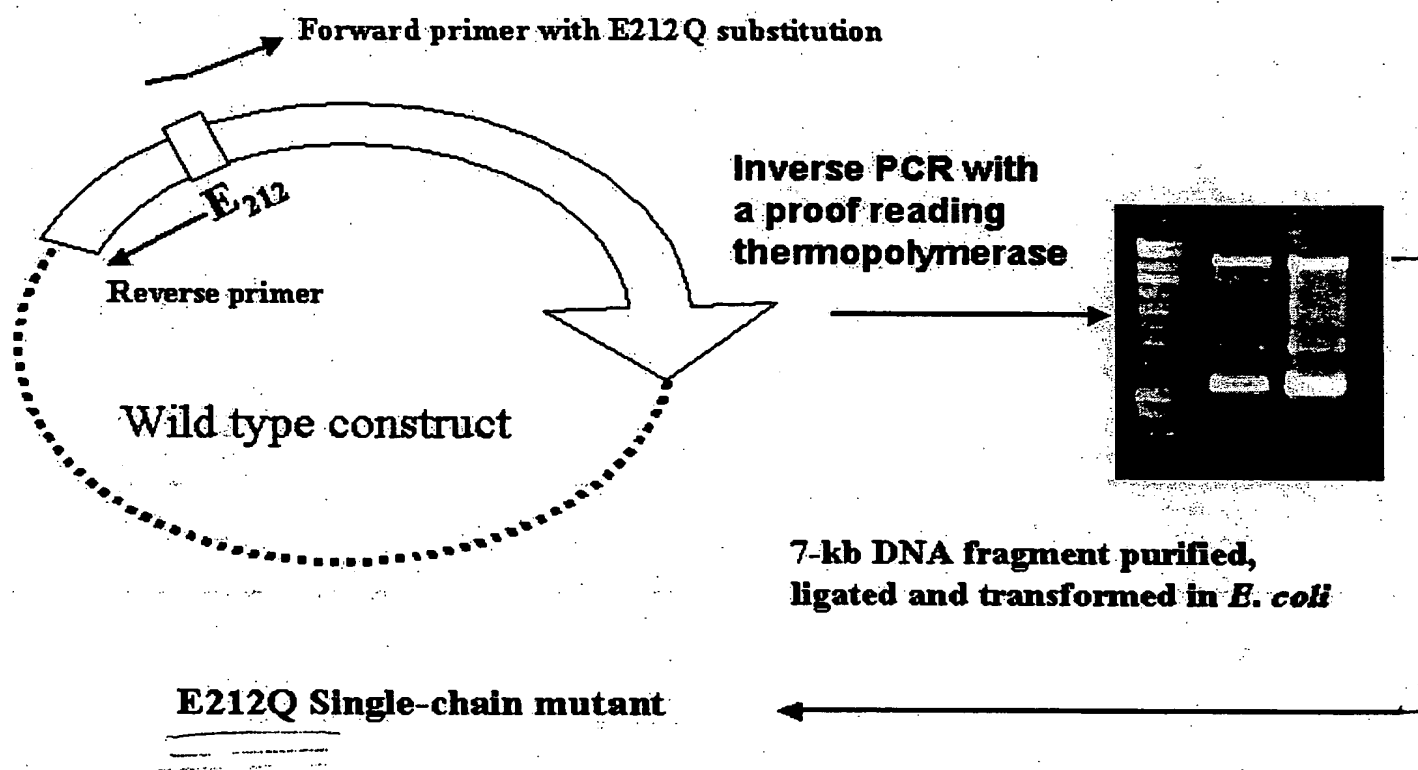
Poly histidine tag

Bam HI

BoNT/E light chain

005280-26984960

Fig. 11



005280-26981960

Fig. 12

Culture of *E. coli* ML5[pQEESCwt]



Overnight induction with IPTG



Harvest, extraction and centrifugation



Metal chelate affinity chromatography

SDS-PAGE (reducing cond.)

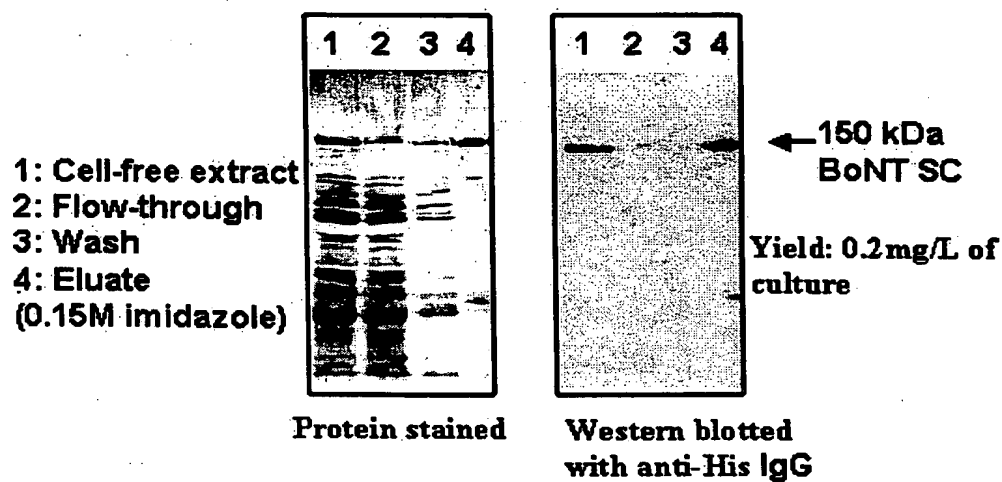


Fig. 13

1: BoNT/E from *Clostridium botulinum*
 2: Un-nicked recomb. BoNT/E
 3: Nicked recomb. BoNT/E
 M: Markers

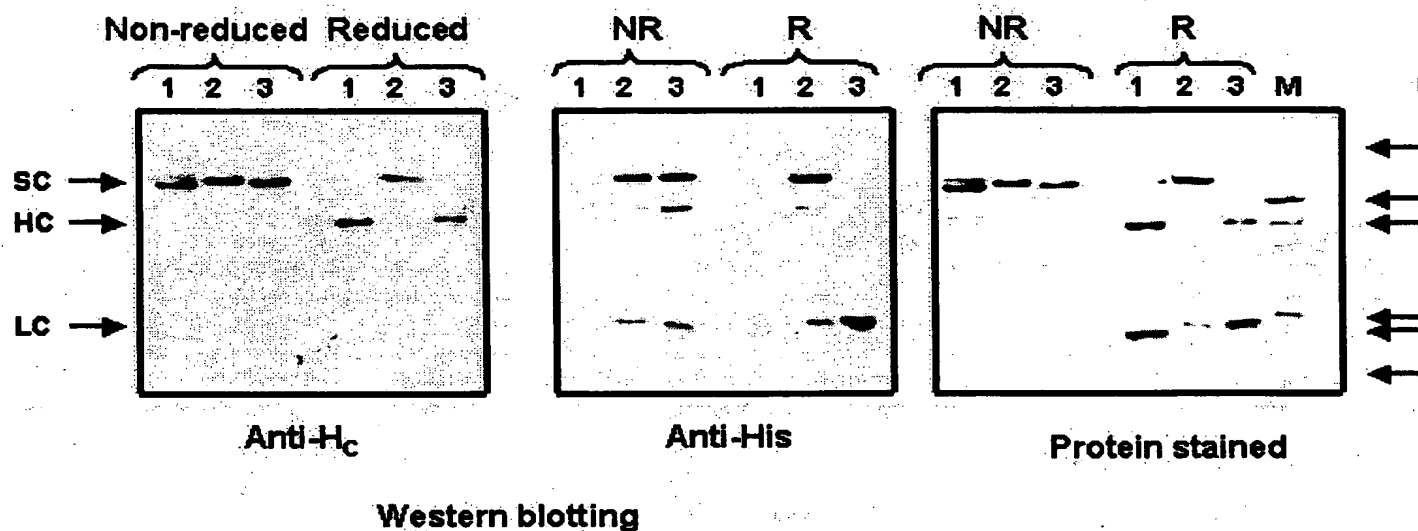


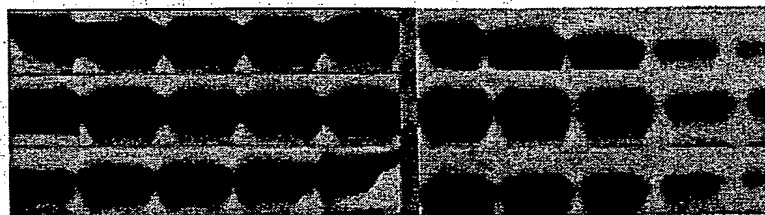
Fig. 14

GST-SNAP-25(140-205)
incubated with:

Native dl - chain
(unlinked) Recomb. single - chain
(unlinked) Recomb. dl - chain

- DTT

+ DTT



(nM) 0.04 0.2 1 5 25 0.04 0.2 1 5 2

Recomb. dl - chain mutant



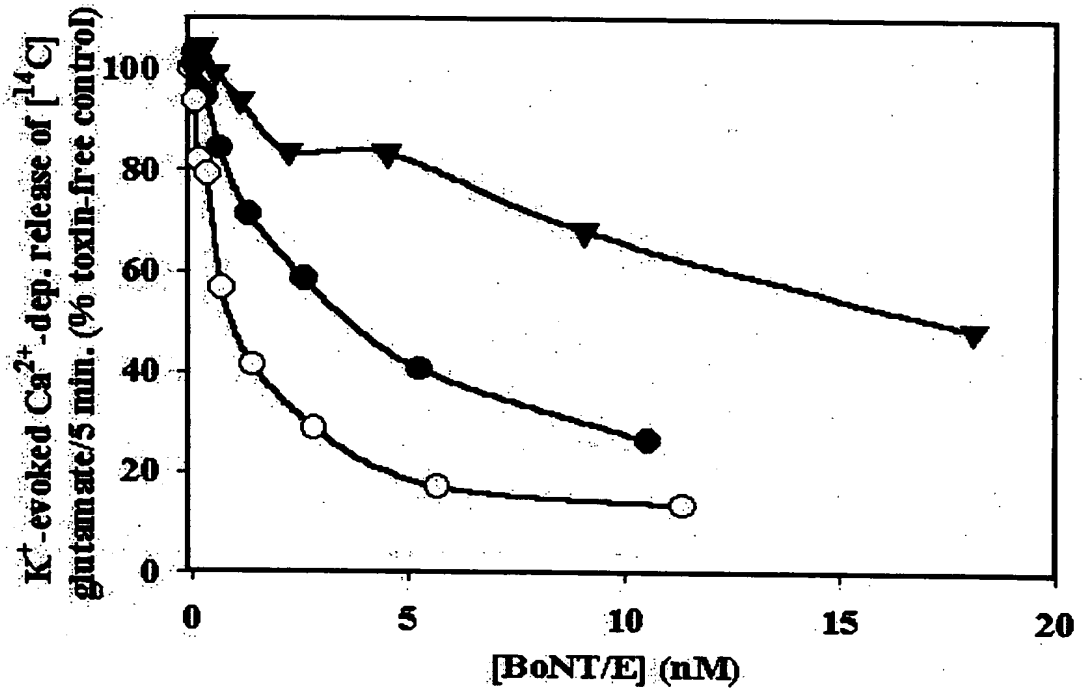
(nM) 125 25 5 1 0.2 125 25 5 1 0.

E 212 Q

proteolytically
deficient.

005280-26384960

Fig. 15



- native
- unlinked 120kDa
- ▼ unlinked 100kDa

Cerebellar neurons.

Fig. 16

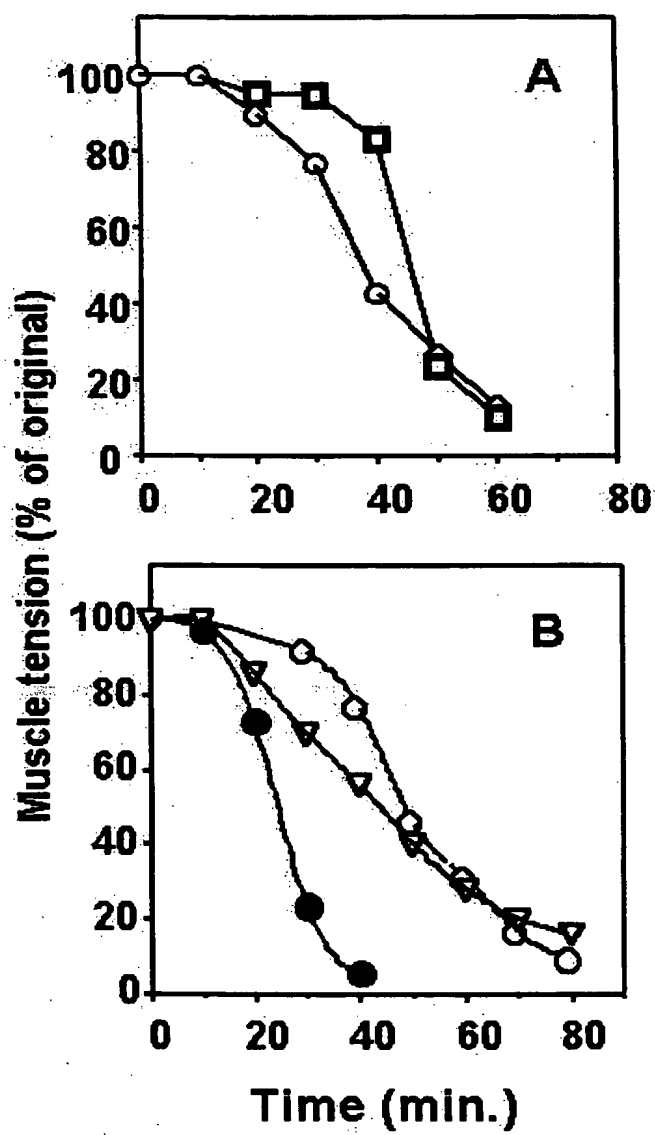
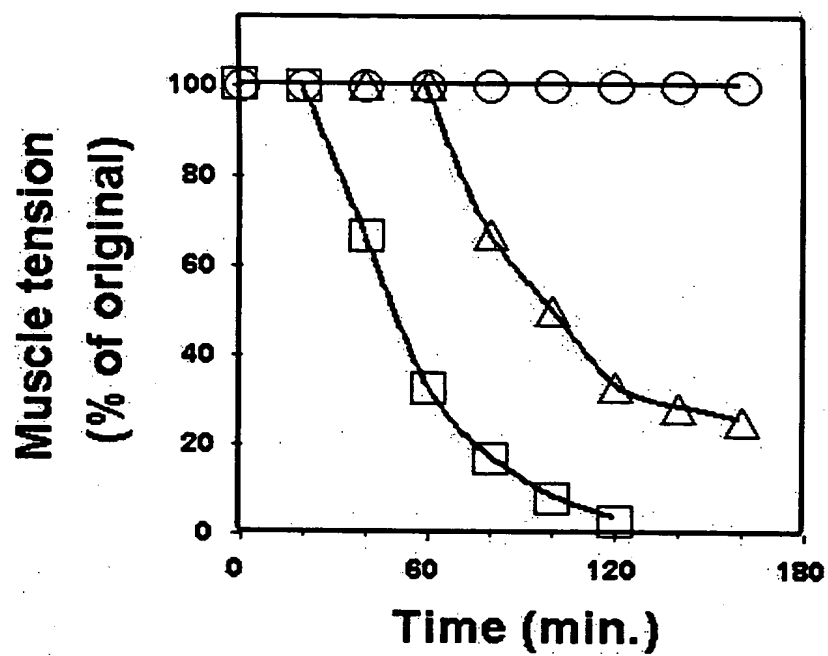


Fig. 17



005260-26984960